Appendix II

Budget Allocation

Personnel Costs: $355,064.27
Fringe: $98,318.04

Subtotal (personnel): $453,382.31

Non-personnel Costs at UNCCH: $601,617.00
Non-personnel costs (NCSU, UNCC, UNCW): $660,000.00

Subtotal (non-personnel): $1,261,617.00

Total: $1,714,999.31

*Per UNC CH Policy, personnel fringe will be paid by the Collaboratory from this award prior to funding transfer.

Budget Summary

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>EHRA Salary</td>
<td>$163,743.47</td>
</tr>
<tr>
<td>SHRA Salary</td>
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<td>Grad Student</td>
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<td>$47,898.30</td>
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<tr>
<td>Fringe Pool</td>
<td>$98,318.04</td>
</tr>
<tr>
<td>Non-Personnel Expenses**</td>
<td>$1,261,617.00</td>
</tr>
<tr>
<td>Total</td>
<td>$1,714,999.31</td>
</tr>
</tbody>
</table>

** Non-personnel line includes $660,000 that will be transferred via subcontract to partners at NCSU, UNCW, and UNCC.
Appendix III

NC Collaboratory Statement of Work:
Title: Tracking SARS-CoV-2 in the Wastewater Across a Range of North Carolina Municipalities

Short title: NC Wastewater Pathogen Research Network (WWTP Path)

Rachel T. Noble (lead PI, UNC-CH), Francis De Los Reyes (coPI, NCSU), Angela Harris (coPI, NCSU), Jill Stewart (coPI, UNC-CH), Larry Cahoon (coPI, UNC Wilmington), Larry Engel (coPI, UNC-CH), Art Frampton (coPI, UNC Wilmington), David Holcomb (coPI, UNC-CH), Sheila Holman (coPI, NC DEQ), Jane Hoppin (coPI, NCSU), Helena Mitasova (coPI, NCSU), Mariya Munir (coPI, UNC Charlotte), Marc Serre (coPI, UNC-CH)

Additional project team collaborators: Steven Berkowitz (NC DHHS), Virginia Guidry (NC DHHS), and Mark Sobsey (UNC-CH)

Funding period: May 4, 2020 through December 31, 2020

INTRODUCTION: The novel coronavirus referred to as SARS-CoV-2 causes severe respiratory distress, therefore its label as a SARS-like virus (Severe Acute Respiratory Syndrome). The actual disease that SARS-CoV-2 causes is referred to as COVID19 (Coronavirus Infectious Disease 19). COVID-19 is caused by this member of the family “Coronaviridae” within the genus Betacoronavirus. Importantly, this group of viruses causes extensive known respiratory distress in the form of the “common cold” as coronaviruses and rhinoviruses combined cause the majority of this disease outcome. Coronaviruses are positive-sense single-stranded RNA viruses, which makes them evolutionarily and biochemically similar to other similar viruses such as norovirus, and human immunodeficiency virus (HIV). SARS-CoV-2 is contagious in humans and is the causative agent of a major pandemic outbreak that initiated in the Wuhan Region of China. At the current time, millions of cases have been counted, with case fatality rates (CFR) that range from less than 1% to as high at 15% in specific populations. A major issue has emerged with limited molecular diagnostic (MDx) testing. Many new tests are approved and being used, but the need for a large-scale tracking approach remains high as we move forward into understanding the ramifications of specific mitigation and social distancing guidelines. Given their role in the clinical environment, SARS-like Coronaviruses have been relatively well-studied in the context of aerosols, hospital and clinical surfaces, and in the context of appropriate disinfectants. PI Noble has been a key participant in early collaborative efforts by an international team of SARS-CoV-2 researchers including Sandra McLellan (Wisconsin), Kyle Bibby (Notre Dame), John Griffith (SCCWRP), Ali Boehm (Stanford), Krista Wigginton (U. Michigan), to name a few. In early March, a subset of this team of international researchers (>45) began to work together to assemble a peer-reviewed publication (Bibby et al., 2020, in review). It was noted early in the pandemic that quantification of the SARS-CoV-2 RNA n wastewater was a key step in conducting wastewater-based epidemiology (WBE, e.g. Medema et al. 2020). It has already been shown that the tools for quantification, if conducted at a high level of technical molecular proficiency are accurate for
understanding the dynamics of the pandemic in a way that is non-invasive and aggregate. The trends and stages of localized community prevalence, or re-emergence of an outbreak. Once established in North Carolina, this surveillance program can coordinate with similar efforts across the United States as an early warning system that is not dependent on clinical testing of COVID-19 infected individuals. This will provide critical information to public health officials as they manage this crisis.

A few key North Carolina research laboratories have combined decades of experience in the use of quantitative molecular techniques such as those currently recommended for use by the CDC and WHO, as well as building relationships with MDx corporations and building capacity for this and future pandemics. The MDx tools that are used for viral pathogen quantification in wastewater are actively implemented with high levels of quality assurance in a few key laboratories in the State of North Carolina, including laboratories of faculty at UNC Chapel Hill (Drs. Rachel Noble and Jill Stewart ) and North Carolina State University (Drs. Francis De Los Reyes, Angela Harris), where expertise on viral pathogen quantification has existed for decades. A large scale WBE approach will be vital for the State of NC for multiple reasons. First, we have the excellent research capacity to conduct high quality quantification and some of the best social mobility and epidemiological researchers in the world to place the generated data into context. Second, a wide range of rural, suburban and urban wastewater treatment plants exist for which to evaluate community prevalence. Specifically given the newly available public case count and hospitalization/death data at the zip code level, this will be a tool to provide non-clinical accuracy to assessments. Third, WBE has been used for decades in different viral pandemics/outbreaks, and can be used to ascertain the total number of undiagnosed, mild and asymptomatic infections which will be critical to refining epidemiological models. Fourth, WBE-based quantification approaches using MDx can be later applied to effluent and sewage discharges, floodwaters (in the case of hurricane and flood related inundation), shellfish, and food products and biosolids. All of these targets will permit quantification of the SARS-CoV-2 pathogen to proactively protect the markets and industries of North Carolina, thereby helping the state to stay on its feet economically. The analysis tools could also provide critical monitoring of transmission at the community level, particularly at nursing homes, and medical facilities, and will support strategies to control community transmission that are emerging as a patchwork.

The overall goal of this research would be to contribute valuable information to the State of NC on the presence and persistence of SARS-CoV-2 like viruses in complex infrastructure and environmental systems as a metric of community COVID-19 prevalence. This information is valuable for the direct protection of the public, but direct data will also assist us in allaying any concerns of the public about the burden of disease in specific areas, and trends associated with clinical approaches and treatments.

OBJECTIVE 1: Develop cross-state collaborative project team to conduct WWTP collection, sampling and analysis relying on a framework of existing collaborative academic-state-industry relationships. To date, 14 municipal WWTP have been enrolled in the study, with active sampling commencing at 8 of these. The objective will be to conduct sampling that is representative of both rural and urban WWTP across at least 6 counties across the state. This geographic scope will be increased as the surveillance system is developed. The project team is interested in conducting specific analyses at the lead universities, and would be interested in contributing this effort to the project if it was of interest. This will include sampling and coordination in collaboration with Drs. Stewart and Noble (UNC Chapel Hill), De Los Reyes and Harris (NCSU), Cahoon and Frampton (UNCW), as well as others to be identified. We will work with members of NC DHHS (Steven Berkowitz) and NC DEQ (Sheila Holman, Danny Smith). We have identified potential other universities that we are interested in collaborating on this project (WCU, NC A & T, UNC Charlotte).
OBJECTIVE 2: Ensure all fully quantitative SARS-CoV-2 and pan-Coronavirus MDx, with accompanying quality assurance and quality control measures, are running in four lead laboratories with full optimization for both qRT-PCR and digital droplet PCR methods. This is a stepwise, vital, and necessary process, and the lead laboratories for this project have decades of experience with training for the use of these tools. Toward this effort, the lead laboratories are participating in several international standardization activities.

OBJECTIVE 3: Collaborate with state and local stakeholders, including NC Department of Health and Human Services, NC Department of Environmental Quality, local health departments, and municipal wastewater utilities, to pilot test a surveillance system that will identify outbreaks, increases in prevalence, or sustained absence of SARS-CoV-2 in a timely manner to support decision making around the COVID-19 response. Stakeholders will help identify possible data sources that may contribute to the success of the surveillance system, as well as strategies for merging and analyzing the data across scales and needs. Stakeholders will also provide technical assistance related to WWTPs and collections systems to inform study design and sampling approaches for both urban and rural systems.

OBJECTIVE 4: Continue to develop and optimize MDx tools from BIOGX and Bio-Rad to build surveillance capabilities for the next decade, and for future pandemics. The lead laboratories have existing collaborations with two multi-national corporations (Bio-Rad, and BIOGX) that will provide valuable support to this project on reagent and equipment availability.

OBJECTIVE 5: Interact with a team of epidemiologists (Dr. Larry Engel, Dr. David Holcomb, Dr. Jane Hoppin, and Dr. Virginia Guidry) and spatial and GIS modeling researchers (Dr. Marc Serre and Dr. Helena Mitasova) to preliminarily investigate the relationships between wastewater concentration of SARS-CoV-2 and contemporaneous/future COVID-19 population prevalence, both in individual populations and aggregated across the study. The factors that influence these relationships will also be studied to the extent possible. If the data permit, we will explore the use of Bayesian Maximum Entropy disease mapping methods to conduct a spatiotemporal mapping analysis of COVID-19 infection to address the small number problem in the context of this project. To achieve these, we will identify the diverse data sources that would contribute to the success of WBE for COVID-19 (and emerging future pathogens) and evaluate the ways to link these data in a timely manner. Included in this effort will be development of a preliminary statewide interactive visualization and mapping tool for spatial and temporal data to support the application of WBE to NC and inform public health decisions. At first, Wake, Orange, Mecklenberg, Carteret, New Hanover, and Durham Counties will be used as model systems to develop a more detailed spatial and temporal model that can ultimately be scaled up to other communities.

Existing Leverage Research Funds and Activities:

A. Noble and De Los Reyes participating in collaborative international group to standardize and validate standard operating protocols for sample collection and sample processing.

B. Noble, in collaboration with Carolina Population Center, funded NSF RAPID COVID-19 project on social mobility utilizing WWTP surveillance data across 3 counties

C. De Los Reyes and collaborators, funded NSF RAPID COVID-19 project on wastewater-based surveillance and temporal patterns of community prevalence.

Proposed Budget, UNC Chapel Hill
May 4, 2020 through December 31, 2020

UNC Chapel Hill: PI Noble $720,000.00
UNC Chapel Hill: CoPI Stewart $255,000.00
UNC Chapel Hill: CoPI Engel $40,000.00
UNC Chapel Hill: CoPI Serre $40,000.00

**Total UNC Chapel Hill Support: $1,055,000.00**

Proposed Budget: NCSU, UNCW, DEQ, UNCC

NCSU: CoPI De Los Reyes $280,000.00
NCSU: CoPI Harris $160,000.00
NCSU: CoPIs Mitasova/Hoppin $85,000.00
UNCW: CoPIs Cahoon/Frampton $75,000.00
UNCC: CoPI Munir $60,000.00

NC DEQ: Holman **$85,000.00 (not included in total below)**

**Total Funds State System External to UNC: $660,000.00**

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<td>UNC CH POOLED BUDGET COVID-19 WWTP</td>
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**TOTAL BUDGET (xfer to Noble) = $1,714,999.31**

*(Table above does not include $660,000 in non-personnel for subcontracts with NCSU, UNCC, and UNCW.*